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Sex differences in the Morris water maze in young rats: temporal dimensions

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There are many factors affecting our memory. The lapse of time between the acquisition and retrieval of information seems to be crucial because the intervening consolidation processes make a lasting engram. In this study, spatial memory was tested in 30day-old male and female Wistar rats by increasing delays between the acquisition phase and the probe test in the Morris water maze. Three groups of female rats were assessed with 1min, 6h and 12h delays as well as a male group tested with a result of a 12h delay. Results of the probe test revealed an adequate performance in males but a tendency in females to search for the missing platform in an incorrect place when long delays had occurred after acquisition (between 6 to 12h delays). These results suggest that females form a weaker representation of the environment than males, and such representation may be altered over time.

Diferencias sexuales en el laberinto acuático de Morris en ratas jóvenes: dimensiones temporales. Existen muchos factores que influyen en nuestra memoria. El lapso de tiempo entre la adquisición y la recuperación de información parece ser crucial. En este estudio se evaluó en el laberinto de Morris la memoria espacial en ratas macho y hembra a los 30 días postnatales, modificando el intervalo entre la fase de adquisición y recuperación. Se utilizaron tres grupos de ratas hembra evaluadas con intervalos de 1 minuto, 6 horas y 12 horas y un grupo de ratas macho evaluadas 12 horas tras la adquisición. La prueba de recuerdo revela una ejecución adecuada en el grupo de ratas macho y una tendencia en las hembras a buscar la plataforma en un cuadrante incorrecto cuando se utilizan intervalos de 6 y 12 horas. Estos resultados sugieren que las hembras forman una representación del entorno más débil, que podría verse alterada más fácilmente que en machos con el paso del tiempo.

Cognitive skills are used to be able to adapt to an ever-changing environment. For thousands of years, spatial memory has contributed to our knowledge and exploration of the available resources in our surroundings. Spatial tasks reveal different abilities in male and female rats, although using different protocols, tasks, species and ages has produced variable results (Williams, Barnett and Meck, 1990; Berry, McMahan and Gallagher, 1997; Warren and Juraska, 1997; Astur, Ortiz and Sutherland, 1998; Roof and Stein, 1999; Cimadevilla, Fenton and Bures, 2001). A valuable approach to sex dimorphisms comes from the use of developmental studies, which allow the correlation between brain growth and behavioural achievements. In this respect, Cimadevilla et al. (2001) showed that an active place avoidance task discriminates between male and female performances in rats by comparing their spatial abilities during the weaning period. Males achieved a correct performance in the task before females: 24 postnatal days and 33 postnatal days for males and females, respectively. Another study reported male superiority at 30 postnatal days in the spatial version of the Morris water maze (MWM) task (Cimadevilla et al., 1999). This difference disappeared when female rats were treated with testosterone propionate at postnatal day 1, which suggests a hormonal- dependant maturation process underlying between the training and testing phases, which raises the question of whether the differences are immediate or if they emerge due to the delay between the acquisition phase and the testing trial.

This study attempts to explain female rat's performance. Spatial orientation of male and female rats was assessed in the MWM by increasing intervals between the acquisition phase and the probe test (at 1 min, 6h and 12h in female rats and at 12h in male rats). Our study found that spatial sex differences emerge at longer delays.

Materials and methods

Subjects

One-month old Wistar rats (30 females and 10 males) from the main vivarium at the University of Oviedo were used. Animals were kept in a 12h light/dark schedule (light on: 08:00-20:00) and housed by sex in groups of five animals with free access to food and water. Animals came from seven different litters. Pups were weaned at 21 days old. Females were assigned randomly to the experimental groups (probe test ran at 1min, 6h and 12h delays). The work was conducted in accordance to the NIH guide for the maintenance and use of animal laboratory.

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Apparatus

A black, circular pool (150 cm-diameter × 40 cm-deep) made out of fibreglass was placed over a metal platform 35 cm high. This was placed in a room (4m × 4m) with several landmarks, where there were eight patterned plates hanging from a white wall in the North, a shelf and a map in the South, a computer and a table where the animal box remained during the experiment in the West and a door and a covered window in the East.

The only light in the room was that produced by two lamps located near the floor in the South and West location, which indirectly illuminated the entire room. The pool was divided conceptually into four quadrants to assess the animal's movement and a black, rounded plastic platform (diameter 10 cm) was placed in the northern quadrant, approximately 30 cm from the wall of the pool. The water level was kept at 1 cm above the surface of the platform and the temperature of the water was maintained between 22 ºC and 23 ºC.

Procedure

The spatial learning task was performed between 9:00h and 15:00h. The animals received two daily training sessions during four consecutive days. The two daily sessions were separated by a 2 hours interval. Each session was comprised of our trials with an inter-trial interval of 30s. A trial consisted of randomly releasing the animal from one out of four compass locations around the pool (North, South, East, West) and allowing the animal to swim until it either came upon the hidden platform (North quadrant) or until 60 seconds had elapsed. The latency to the platform was recorded. If an animal failed to find the platform within 60 seconds, it was taken back there for 15s. During the inter-trial interval the animals were placed inside a black bucket. They were returned to their home cages between sessions.

A probe test was run after the last trial of day 4. Before the probe test, the hidden platform was removed, then the rat was introduced from the quadrant opposite to the escape quadrant and it was left to search for the platform for 60s. Each probe trial was recorded with a camera located on the ceiling of the room and the recording transferred to the computer for further analysis. The total length of the path, the time spent in each pool quadrant and the time spent swimming near the walls (in a 20 cm alley) during the probe test were all quantified. Probe trials were run with 1 min, 6h and 12h delays for the female rats (only once in each experimental group). Males were assessed with a 12h delay.

Results

The time spent to locate the escape platform was grouped according to days (2 sessions per day, 4 trials per session) in order to apply statistical tests. During the first trial rats swam close to the walls hardly exploring the middle of the pool at all. However, this situation changed radically after a few trials when the animals began to make contact with the platform. The thigmotaxic behaviour disappeared and consequently a progressive reduction in latencies occurred. ANOVA (animal × group × day) with repeated measures for the last factor revealed the absence of differences between groups in the latency to reach the platform (females tested with 1min, 6h and 12h delays and males with a 12h delay) ($F_{3,36}$= 1.76, $p<0.05$), however it showed a significant main effect of days ($F_{3,108}$= 143.5, $p<0.001$). Post-hoc the test showed a continuous and significant reduction in the time needed to reach the platform from one day to the next ($p<0.05$) (Fig. 1).

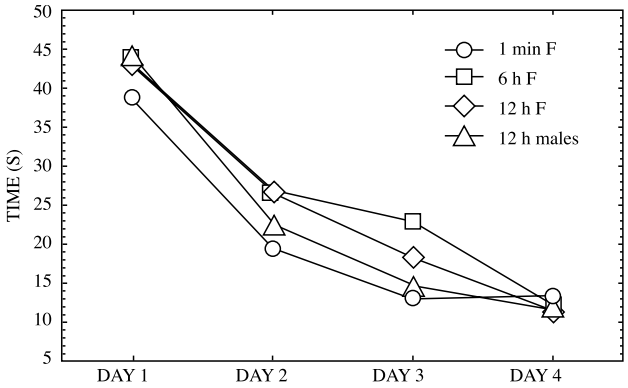

Fig. 1. *Latencies to find the hidden platform during the four days of training in the pool. Note that on day 4 all the groups reached the same level*

On the other hand, the time spent in each virtual quadrant of the MWM during the probe test was analysed by repeated measures ANOVA (animals × quadrants) for each group. It is usually assumed that subjects achieve an adequate performance if they swim significantly longer in the escape quadrant in comparison to the other three quadrants. For result analysis, the first and second 30s periods of the probe test were considered separately.

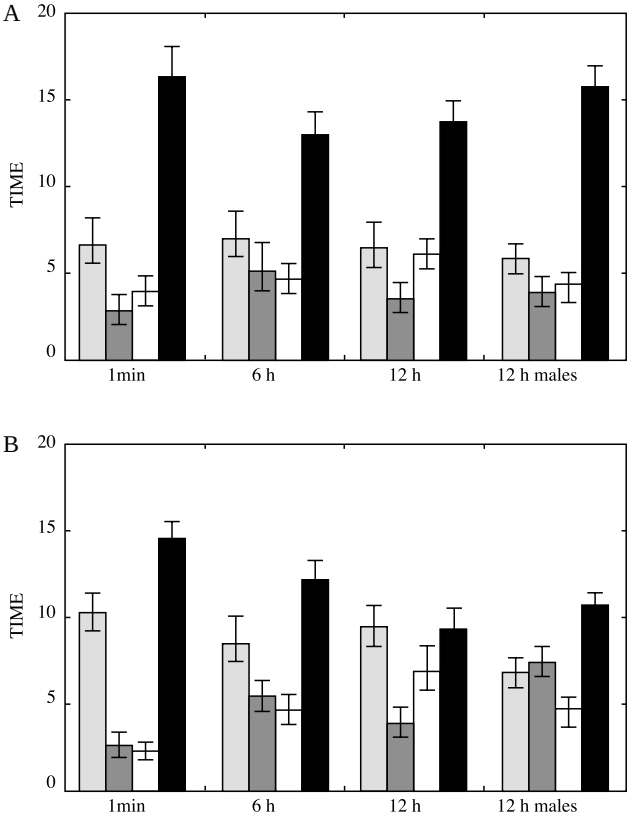

Fig. 2. *Time spent in the four virtual quadrants during the probe test. The black column represents the escape quadrant. A) Initial 30 s period. All groups preferentially searched the platform in the escape quadrant. B) Second 30 s period. Females evaluated at 6 h and 12 h delays searched into two quadrants for an equal time. This did not occur in males. Mean ± SEM*

During the first 30s period ANOVA showed a significant main effect of quadrants in all the groups ($F_{3,27}= 17.1$, $p<0,05$; $F_{3,27}= 6.05$, $p<0.05$; $F_{3,27}= 11.7$, $p<0.05$ and $F_{3,27}= 28.01$, $p<0.05$) for females tested with delays of 1min, 6h 12h delays and males with a 12h delay, respectively .The post hoc test revealed an extended search in the escape quadrant for all the groups (Fig. 2A). After analysing the last 30s period, a significant main effect of quadrants also appeared in all the groups ($F_{3,27}= 35.02$, $p<0.05$; $F_{3,27}= 7.58$, $p<0.05$; $F_{3,27}= 3.2$, $p<0.05$ and $F_{3,27}= 7.64$, $p<0.05$). Post-hoc analysis showed a bias towards the escape quadrant in male rats and in female rats with a 1 min delay ($p<0.05$). Female rats tested at 6h and 12h intervals showed equal preference for the escape and western quadrants (Fig. 2B).

Additionally, the total distance covered during the probe test was analysed by a one-way ANOVA, which revealed significant differences between groups ($F_{3,36}= 6.64$, $p<0.05$). Post-hoc analysis (Tukey's test), showed that groups tested with 12h delays displayed shorter paths than groups with 1 min and 6h delays ($p<0.05$).

Finally, in order to establish whether stress could explain these differences by causing female rats to swim close to the walls during the last 30s period in the probe test, in the groups with a 12h delay we compared the time swimming in a 20cm wide ring close to the wall. A Student's t-test revealed no significant difference between male and female rats ($t(18)= 1.55$, $p<0.05$).

Discussion

Memory is a cognitive process that can be studied throughout the life span. Developmental studies can provide a different perspective when approaching memory. This experiment examined the sex–related differences in spatial memory using different time delays between acquisition of spatial information and its retrieval in the probe test. Results indicate that young female rats can perform adequately in the probe trial if they are tested immediately after acquisition or if the time considered for analysis is not longer than 30s for delays until 12h. (i.e. female rats performed well during the first 30s of the probe test). On the contrary, when the second 30s period of the probe trial was considered for analysis, female groups evaluated from 6h or 12h delays showed incorrect searching patterns and spent equal time swimming in two virtual pool quadrants (escape quadrant- northern quadrant- and western quadrant).

On the other hand, the length of the path in the pool during the probe test did not differ in male and females rats tested with a 12h delay, which indicates that the main difference in both sexes does not depend on their levels of activity, an important observation that has also been mentioned by other authors (Bucci, Chiba and Gallagher, 1995).

It is interesting to note that the biased searching displayed by females al long intervals cannot be interpreted as a better strategy by non-perseverance of their response, i.e. a more flexible performance. If this were the case, the time these animals spent swimming outside the escape quadrant would be equally distributed between the three other quadrants and this is not the case. When we consider the whole time outside the escape quadrant, females tested at 6h and 12h delays, spent 48% swimming in the western quadrant and only 25% and 27% in the eastern and southern quadrants, respectively.

This reveals a marked preference for one of the non-escape quadrants. In fact, it is possible to find a relationship between a reduction in reference memory in 30 day-old female rats and this bias towards a quadrant different from the escape one. This preference for another quadrant is a time-dependant phenomenon, i.e. it only appears with long delays. Long intervals between acquisition and retrieval cause impairment and females persist in looking for the platform in places that possibly offered them misleading cues.

Our results confirmed those reported previously, in which 30day-old female rats gave a poorer performance than males in the spatial version of the MWM with a 24h delay (Cimadevilla et al., 1999). It is necessary to point out the temporal condition of these differences in developmental studies. In a previous study, when subjects had to avoid in a spatial task a punished area fixed to distal cues, male rats outperformed female rats between 23 and 30 postnatal days if the delay between acquisition and retrieval was 24 hours. However, when no temporal parameters were considered no differences between sexes were found (Cimadevilla et al., 2001). According to different studies, the first 24 hours after training could be important to construct a representation of the context (Rudy and Morledge, 1994; Wallenstein, Vago and Walberer, 2002) and also to consolidate memories (Bohbot, Otáhal, Liu, Nadel and Bures, 1996; Barrientos, O'Reilly and Rudy, 2002; Stupien, Florian and Roullet, 2003).

The differences found in our work could be due to the adoption by females of less effective orientation strategies such as single associations between the locations occupied by the platform and room cues or by the use of weak representations of the environment. The first hypothesis is in agreement with other studies (Williams et al., 1990; Roof and Stein, 1999) that suggest the different use of room landmarks by male and female rats. It was hypothesized that non-geometric information is subordinate to the use of the shape of environment in male rats. Instead, female rats learn to use two cues: landmarks and geometry (Williams et al., 1990). In addition to this, it was demonstrated also in humans the differential use of cues in a virtual environment by manipulating the availability of geometric cues and landmarks (Sandstrom, Kaufman and Huettel, 1998).

The second hypothesis that could justify our results points to a problem in consolidation in female rats which would use weak representations and more labile memories of the context. The temporal condition of the differences found, as well as another studies (Cimadevilla et al., 1999; Cimadevilla et al., 2001) support this statement. In addition, several morphological and physiological changes in the hippocampus, a brain structure involved in spatial memory consolidation affect young male and female rats differently (Madeira, Sousa and Paula-Barbosa, 1991; Maren et al., 1994; Madeira and Lieberman, 1995; Vicens, Redolat and Carrasco, 2003) and it is well known that gonadal steroids can affect hippocampal morphology, mnemonic functions and spatial abilities (Haaren and Hest, 1990; Galea, Kavaliers, Ossenkopp, Innes and Hargreaves, 1994; Isgor and Sengelaub, 1998; Isgor and Sengelaub, 2003; Naghdi, Oryan and Etemadi, 2003).

Recently it was reported that androgens affect CA3 field differentially, regulating developmental events such as somal growth and neuronal differentiation that contributes significantly in producing the sex difference in spatial abilities between male and female rats (Isgor and Sengelaub, 2003). In addition to this, the blockage of CA3 region was demonstrated to affect consolidation processes in a spatial task (Stupien et al., 2003) and the hippocampal regions were reported to contain different numbers of astroglia in young male and female rats, which can be related to

the synaptic activity and functionality of this brain region (Conejo, González-Pardo, Cimadevilla, Vallejo-Seco and Arias, 2003). Obviously, we can not discard an interaction of those factors that account for both hypothesis above mentioned (i.e. interaction between a different use of cues and weaker consolidation processes in female rats). A deeper knowledge about maturational and hormonal factors that determine brain development could help to explain the different behaviours shown by males and females as well as the more general processes underlying learning and memory.

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